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ENTHALPY CHANGES OF SALTING PROCESSES OF HEN-EGG WHITE LYSOZYME IN VARIOUS ELECTROLYTE SOLUTIONS

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The enthalpy changes of salting process of hen-egg white lysozyme in buffer acetate solutions (pH=4.25) as a function of concentration of following electrolytes: LiCl, KCl, K_2SO_4, Li_2SO_4 and $(NH_4)_2SO_4$ are determined. Obtained data according to McMillan and Mayer's approach, has been analyzed in the terms of the enthalpic pairwise interaction coefficients: lysozyme – lysozyme h_{xx}, and lysozyme – salt h_{xy}. The ability of cations to precipitate lysozyme solution in relation to the concentration of cations can be seen from the series as follows: $Li^+ > Na^+ > K^+ > NH_4^+$.

Keywords: *enthalpy of salting, enthalpic pairwise interaction coefficients, lysozyme, titration calorimetry*

Introduction

Interactions between proteins and ions play an important role in a number of physiological and biochemical processes. Salts with various ions are often used to modify conformational stability, equilibrium solubility of biological macromolecules [1]. Salts are also used in study of the precipitation, crystallization and salting process of proteins. Microcalorimetry is the method frequently used in different proteins study [2–5].

In our previous work [2] have been reported the results of the determination of the changes of the enthalpy of lysozyme with increase of NaCl concentration. It was found that: (a) the salting process is connected with negative enthalpy changes; (b) the increase of salt concentration enlarge the negative value of the enthalpy; (c) the enthalpy changes could be represented as a monotonic function of the salt concentration. However there exists the region of NaCl concentration in which are observed deviations from monotonic decreasing of the enthalpy value. This deviation was observed in the range of 0.5–0.7 M of NaCl concentration. At this range concentration lysozyme aggregation begins [3, 6].

In order to enrich our knowledge about the salting process of the lysozyme we decided to enlarge our thermochemical investigations of salting process of lysozyme by analyzing the ability of lithium chloride, potassium chloride, lithium sulfate, potassium sulfate and ammonium sulfate to precipitate lysozyme solution in relation to the concentration of these salts.

Experimental

Lyophilized, dialyzed and salt free lysozyme (Cat. no. 62970), lithium sulfate (no. 62613), lithium chloride (no. 62476), ammonium sulfate (no. 09978), potassium chloride (no. 60128) potassium sulfate (no. 60528) were purchased from Fluka Co.

Before the measurements the lysozyme powder was dissolved in 0.1 M acetic/NaOH buffer (pH 4.25), filtered through 0.22 μm syringe filter (Roth, Germany) and degassed. The pH value of the buffer as well as of the lysozyme stock and NaCl buffered solutions were adjusted independently (±0.05 pH) on a Mettler Toledo pH-meter (MP 220). The protein concentration was determined by UV absorption spectrum, assuming the extinction coefficient ε_{280}=38220 M^{-1} cm^{-1}.

ITC measurements were carried out at 25°C on a Microcal Omega ultrasensitive titration calorimeter (MicroCal Inc.). The parameters of the titration (the number, volume and length of time of injections) are input into the software program controlling data. The electrolyte buffered solutions in the cell were stirred by the syringe at 400 rpm. The volume of a titrant (15 μL) was injected over 30 s with an interval of 240 s between injections from a 250 μL injection syringe (containing lysozyme) into the sample cells in a series of several controlled pulses to the final concentration of lysozyme of about 0.001 M. The sample cell volume is 1.3611 cm^3. Such final concentration of lysozyme is the one, which renders crystallization condition suboptimal. In this concentration lysozyme exists as a monomer only [7] which makes it possible to analyze the effect introduced by changing the electrolyte concentration.

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The output from calorimeter control system comprised the rate of heating required to rebalance the temperatures of sample and reference cells as a function of time following each pulse. The software used these data to produce a plot recording heat $q(l)$ as a function of injection number l. The integrated heat effects of each injection were corrected by subtraction of the corresponding integrated heat effects of lysozyme injection to the pure buffer and heat effects of buffer injection to the salt solution.

The total enthalpy of salting involved in the series of l succeeding injections was evaluated according to a relation given below:

$$\Delta_{sal} H_m(l) = \frac{\sum_{l=1}^{l} \Delta H_m(l)}{\sum_{l=1}^{l} n(l)} \quad (1)$$

where $\Delta H_m(l)$ is the experimental enthalpy arising from mixing adequate concentrations of lysozyme and salt in the l^{th} injection; $n(l)$ is the number of moles added to the cell in the l^{th} injection.

The enthalpy of salting data was also performed according to the McMillan–Mayer [8, 9] approach in the terms of enthalpic pairwise interaction coefficients and calculated according to the Eq. (2). The procedure followed in [2] is similar to the one adopted in the present manuscript.

$$\Delta_{sal}H_m(m_x,m_y)=h_{xx}(m_x)^2+h_{yy}(m_y)^2+h_{xy}(m_x,m_y) \quad (2)$$

The molarity of the lysozyme x and salt y are expressed in number of moles per kilogram of solvent [M], i.e., m_x, and m_y, respectively. Any change in the lysozyme – lysozyme, salt – salt, lysozyme – salt interactions are incorporated in the enthalpic pairwise interaction coefficients, h_{xx}, h_{yy}, h_{xy}, respectively.

Results and discussion

The determination of the changes of the enthalpy of salting with concentration of salts was performed by doing several measurements for each salt at different concentrations. In each of those measurements 16 injections were made. For example, values of enthalpy $\Delta H_m(l)$ arising from mixing solutions of lysozyme with ammonium sulfate of various concentrations are presented in Table 1, where m_y is the initial concentration of salt in the cell. The initial concentration of lysozyme in the syringe was practically the same, about 0.007 M.

The enthalpies of salting $\Delta_{sal}H_m$ of chloride and ammonium salts calculated according to Eq. (1) are presented in Tables 2 and 3. From the experimental data it can be stated that salting process is connected with negative enthalpic values. The increase in salt concentration increases the negative value of the enthalpy. The changes of $\Delta_{sal}H_m=f(m_y)$ could be represented as a monotonic function of the salt concentration, however, there exist a region of salt concentrations in which we can observe deviations from monotonic decreasing of the enthalpic values. These deviations could be attributed to specific salting pro-

Table 1 The enthalpic values arising from mixing solutions of 0.007 M of lysozyme and ammonium sulfate of various concentrations

	m_y/M									
k	0.2010	0.3016	0.4008	0.5004	0.6009	0.7007	0.8003	0.9009	1.0014	1.2001
	$-\Delta H_m(l)$/μJ									
1	2567.9	2951.3	3907.6	3000.9	4418.5	5400.4	5566.0	5245.6	5946.6	7043.1
2	4957.4	5749.6	7657.3	7026.5	8682.1	10466.7	11175.7	10368.2	10892.1	14358.5
3	7336.3	8564.4	11376.3	11031.7	12959.5	15827.4	16762.6	15538.6	16037.0	21469.9
4	9657.7	11308.6	15017.6	14951.7	17185.9	21136.2	22245.9	20678.9	21097.9	28393.9
5	11917.6	13978.3	18576.7	18787.6	21316.3	26402.7	27672.8	25734.5	26147.4	35300.7
6	14118.3	16600.5	22044.1	22564.0	25376.5	31553.6	32957.0	30637.4	31053.8	42096.3
7	16316.3	19202.4	25499.4	26281.0	29416.9	36644.3	38196.0	35431.9	35965.6	48897.7
8	18432.5	21707.9	29017.4	29883.2	33372.7	41588.3	43335.2	40243.0	40746.2	55595.2
9	20486.0	24144.1	32305.3	33413.8	37231.5	46406.5	48368.4	45005.1	45417.6	62079.4
10	22516.2	26538.9	35489.3	36905.8	41052.9	51129.9	53311.7	49706.0	50064.9	68669.9
11	24494.2	28899.7	38551.6	40322.4	44814.5	55821.6	58135.7	54278.3	54659.2	75166.7
12	26425.2	31221.4	41624.6	43667.1	48514.9	60494.4	62902.2	58820.0	59132.6	81514.5
13	28329.6	33506.6	44651.7	46967.2	52149.7	65058.9	67607.8	63300.1	63558.4	87798.0
14	30185.7	35752.0	47627.1	50203.2	55734.3	69571.1	72249.6	67717.8	67904.6	94026.5
15	32003.7	37921.7	50514.2	53361.5	59224.0	74004.0	76743.2	72025.9	72211.3	100205.0
16	33796.0	40074.3	53359.7	56471.3	62675.5	78266.1	81090.7	76262.0	76468.7	106418.0

Table 2 The enthalpy of salting $\Delta_{sal}H_m$ for chloride salts

Sodium chloride*		Lithium chloride		Potassium chloride	
m_y/M	$\Delta_{sal}H_m$/kJ mol^{-1}	m_y/M	$\Delta_{sal}H_m$/kJ mol^{-1}	m_y/M	$\Delta_{sal}H_m$/kJ mol^{-1}
0.3001	–23.89	0.2009	–10.27	0.2007	–13.13
0.3512	–25.73	0.2514	–11.01	0.3003	–18.65
0.4009	–29.14	0.3027	–14.70	0.4009	–23.27
0.5004	–35.27	0.4163	–14.56	0.5012	–29.01
0.6010	–46.05	0.5006	–14.32	0.6005	–36.89
0.6531	–42.61	0.6006	–11.94	0.7007	–41.71
0.7003	–42.11	0.7001	–12.39	0.8001	–38.07
0.7246	–43.07	0.8016	–12.39		
0.7517	–43.16				
0.7740	–48.23				
0.8049	–58.03				
0.8547	–59.85				
0.9012	–63.68				

*non-published data of. J. Poznański, M. Wszelaka-Rylik and W. Zielenkiewicz

Table 3 The enthalpy of salting $\Delta_{sal}H_m$ for sulfate salts

Ammonium sulfate		Potassium sulfate		Lithium sulfate	
m_y/M	$\Delta_{sal}H_m$/kJ mol^{-1}	m_y/M	$\Delta_{sal}H_m$/kJ mol^{-1}	m_y/M	$\Delta_{sal}H_m$/kJ mol^{-1}
0.2010	–20.12	0.2029	–25.05	0.2000	–12.41
0.3016	–23.85	0.3008	–33.08	0.3012	–14.76
0.4008	–31.55	0.4011	–40.32	0.3507	–19.96
0.5004	–33.61	0.5031	–43.68	0.4497	–27.98
0.6009	–37.31	0.6019	–47.95	0.5012	–24.36
0.7007	–44.23			0.6006	–23.39
0.8003	–48.27			0.7000	–19.08
1.0014	–45.51			0.8501	–17.94
1.2001	–63.34				

cess of lysozyme as was presented in Fig. 1 for lithium sulfate. It was assumed that minimum of the curve (m_y=0.4497 M; $\Delta_{sal}H_m$= –27.98 kJ mol^{-1}) corresponds to the beginning of salting process.

For other salts these minima can be observed for LiCl (m_y=0.3027 M; $\Delta_{sal}H_m$= –14.70 kJ mol^{-1}), NaCl (m_y=0.6009 M; $\Delta_{sal}H_m$= –46.05 kJ mol^{-1}), KCl (m_y=0.7007 M; $\Delta_{sal}H_m$= –41.71 kJ mol^{-1}), $(NH_4)_2SO_4$ (m_y=0.8003 M; $\Delta_{sal}H_m$= –48.27 kJ mol^{-1}). For K_2SO_4 minimum is not defined due to low solubility of the salt above 0.7 M. Thus the obtained series of ability of cations to precipitate lysozyme is as follows: $Li^+ > Na^+ > K^+ > NH_4^+$, which series is in a good agreement with Hofmeister series [10–12]. The analysis of the obtained experimental data of enthalpy of salting was also performed in terms of pairwise interaction coefficients. The pairwise enthalpic interaction parameters h_{xx}, h_{xy} are reported in Tables 4 and 5.

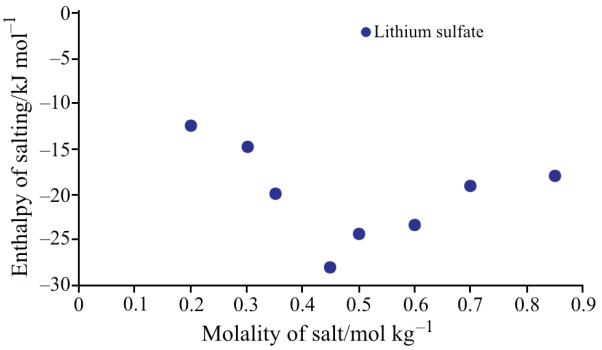

Fig. 1 The enthalpy of salting lysozyme solution expressed as a function of initial lithium sulfate concentration in the sample cell

The interactions between lysozyme-lysozyme pairs are thermochemically favorable. Attraction forces, which result in the observed negative values h_{xx} dominate over the interaction between lysozyme

Table 4 The calculated values of h_{xx}, h_{xy} for the pairs interaction of lysozyme – lysozyme and lysozyme – salts for sulfate salts

Ammonium sulfate			Potassium sulfate			Lithium sulfate		
m_y/M	h_{xx}/J M^{-2}	h_{xy}/J M^{-2}	m_y/M	h_{xx}/J M^{-2}	h_{xy}/J M^{-2}	m_y/M	h_{xx}/J M^{-2}	h_{xy}/J M^{-2}
0.2010	–2577±32	–79.9±0.1	0.2029	–266±113	–73.4±0.5	0.2000	–1457±75	–50.4±0.3
0.3016	–3391±56	–62.8±0.1	0.3008	–2613±111	–95.3±0.5	0.3012	–1795±64	–39.8±0.2
0.4008	–4316±127	–63.4±0.2	0.4011	–5525±234	–115.3±0.8	0.3507	–3299±133	–44.7±0.3
0.5004	–5212±41	–53.7±0.1	0.5031	–10155±191	–138.7±0.3	0.4497	–1635±194	–33.3±0.3
0.6009	–6741±40	–47.8±0.1	0.6019	–14317±181	–148.7±0.4	0.5012	–2468±113	–40.1±0.2
0.7007	–8981±61	–49.4±0.1				0.6006	–3982±79	–30.4±0.1
0.8003	–10967±89	–54.92±0.1				0.7000	–6803±254	–17.3±0.3
1.0014	–8757±140	–34.3±0.1				0.8501	–3763±82	–15.9±0.1
1.2001	–14021±163	–38.9±0.1				1.2010	–10840±296	–25.5±0.2

Table 5 The calculated values of h_{xx}, h_{xy} for the pairs interaction of lysozyme – lysozyme and lysozyme – salts for chloride salts

Potassium chloride			Lithium chloride		
m_y/M	h_{xx}/J M^{-2}	h_{xy}/J M^{-2}	m_y/M	h_{xx}/J M^{-2}	h_{xy}/J M^{-2}
0.2007	–1059±129	–54.4±0.5	0.2009	–590±64	–33.3±0.2
0.3003	–2988±123	–63.5±0.3	0.3027	–711±24	–31.1±0.1
0.4009	–2890±119	–46.8±0.2	0.4163	–1223±24	–23.2±0.1
0.5012	–3845±129	–46.4±0.2	0.5006	–1353±49	–20.5±0.1
0.6005	–5132±167	–48.9±0.2	0.6006	–1300±210	–12.5±0.2
0.7007	–5700±392	–47.6±0.4	0.7001	–1464±34	–11.1±0.1
0.8001	–6004±117	–37.2±0.1	0.8016	–1450±111	–10.5±0.1
0.9008	–8354±121	–47.1±0.9	0.9002		

and salts. The negative values of the enthalpic pair interactions coefficients h_{xx} indicating that the net interactions between buffered lysozyme molecules are attractive. As concentration of salt rises, the enthalpic pair interaction coefficients h_{xx} becomes more negative and the interactions between lysozyme molecules become more attractive, as is typical for salting conditions where the increase in attraction is due to the dehydration of the polar surface of the protein. Thus, aggregation process proves strong concentration dependence of salt.

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